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REVIEW ARTICLE

Current and Future Issues in the Development of Spinal Agents for the Management of Pain

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Abstract: Targeting analgesic drugs for spinal delivery reflects the fact that while the conscious experience of pain is mediated supraspinally, input initiated by high intensity stimuli, tissue injury and/or nerve injury is encoded at the level of the spinal dorsal horn and this output informs the brain as to the peripheral environment. This encoding process is subject to strong upregulation resulting in hyperesthetic states and downregulation reducing the ongoing processing of nociceptive stimuli reversing the hyperesthesia and pain processing. The present review addresses the biology of spinal nociceptive processing as relevant to the effects of intrathecally-delivered drugs in altering pain processing following acute stimulation, tissue inflammation/injury and nerve injury. The review covers i) the major classes of spinal agents currently employed as intrathecal analgesics (opioid agonists, alpha 2 agonists; sodium channel blockers; calcium channel blockers; NMDA blockers; GABA A/B agonists; COX inhibitors; ii) ongoing developments in the pharmacology of spinal therapeutics focusing on less studied agents/targets (cholinesterase inhibition; Adenosine agonists; iii) novel intrathecal targeting methodologies including gene-based approaches (viral vectors, plasmids, interfering RNAs); antisense, and toxins (botulinum toxins; resniferatoxin, substance P Saporin); and iv) issues relevant to intrathecal drug delivery (neuraxial drug distribution), infusate delivery profile, drug dosing, formulation and principals involved in the preclinical evaluation of intrathecal drug safety.

Keywords: Adenovirus transfection, dorsal horn, neurotoxins, pain pathways, spinal drug delivery, spinal analgesics, toxicity.

RATIONALE

Targeting analgesic drugs for direct spinal delivery reflects the fact that while the conscious experience of pain is mediated supraspinally, input initiated by high intensity stimuli, tissue injury and/or nerve injury is encoded at the level of the spinal dorsal horn. Thus, high intensity (*e.g.*, nociceptive) stimulation activates populations of small primary afferents, generating an intensity-dependent increase in activity of second order dorsal horn projection neurons. This excitation is transmitted through spinofugal projection pathways to higher centers, such as the somatosensory thalamus – somatosensory cortex, and to the medial thalamus - limbic cortices that are respectively believed to underlie the sensory-discriminative and affective-motivational components of the pain experience [1-3]. The dorsal horn encoding process reflects a remarkable plasticity wherein the input-output function of the spinal dorsal horn is subject to pronounced regulation by local neuronal and non-neuronal circuits as well as supraspinal (bulbospinal) input. Thus, following tissue or nerve injury there is an enhanced neuronal response to moderate or low intensity stimuli, *e.g.*, a right shift in the stimulus intensity-neuronal response curve. Accordingly, the spinal outflow generated by a given stimulus of moderate or low intensity following tissue and nerve injury generates an enhanced activation of supraspinal systems [4-6] and an enhanced pain state, *e.g.*, hyperalgesia and allodynia [7], respectively. Accordingly, regulation of the spinal input-output function can alter the supraspinally organized pain experience. The organization of the input-output function of the spinal dorsal horn and its associated pharmacology has been the subject of extensive investigation and review [8, 9]. The associated pharmacology of this processing as defined by studies of spinal physiology and behavior after spinal drug delivery has permitted targeting drugs at specific components to alter the content of the spinofugal transmission. While many analgesic drugs may be given systemically to exert their spinal action, the specific use of the intrathecal or epidural route for analgesic drug delivery may be employed for three reasons: i) the drug target lies at the spinal level (opiates, baclofen); ii) the agent does not penetrate the blood brain barrier (antisense, toxins); and/or iii) the agent penetrates the blood brain barrier but does so at systemic doses that produce undesirable side effects, so spinal delivery increases the therapeutic index (*e.g.*, baclofen and opiates).

The efficacy of spinal drugs administered for the management of pain syndromes is dependent on the pharmacology of the underlying mechanisms that mediate

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the particular pain state. Pain states can be broadly categorized as those initiated by an acute noninjurious stimulus, those arising from tissue injury and inflammation and those secondary to nerve injury. Although the source of the pain state may be distinguishable, many clinical pain states likely reflect a combination of mechanisms; and, increasing evidence has pointed to overlapping components at the level of the primary afferent and spinal dorsal horn. These mechanisms and their associated pharmacology and biology have been extensively reviewed elsewhere [10, 11]. In this discussion we will focus on the lumbar intrathecal route of drug delivery and consider: i) ongoing developments in the pharmacology of spinal therapeutics focusing on agents/targets that have been employed and shown to a varying degree to have efficacy in humans, ii) novel targeting methodologies and iii) several issues relevant to intrathecal drug delivery.

SPINAL THERAPEUTIC TARGETS

Spinal analgesic agents may target the primary afferent, the second order neurons, bulbospinal projections and non-neuronal components. From a practical standpoint, many of these pharmacologic targets lie within the parenchyma of the dorsal horn and emphasize that after intrathecal delivery, the topically delivered agents (such as opiates, alpha2 agonists, targeted toxins, and transfection agents targeting 2nd order neurons) must penetrate from the CSF through the pia and then up to several hundred microns in the parenchyma or more to reach these dorsal horn sites to exert their action. These issues will be discussed further below. In other cases, the agent may target the dorsal root ganglion cell (*e.g.*, antisense, viral vectors). This target lies distal to the dural cuff formed by the meninges [12, 13] and is consistent with the fact that the DRG lies outside the blood brain barrier as evidenced by movement of large molecules into the DRG from the blood [14] and by the sympathetic innervation of the DRG vasculature [15]. The necessity to move from the CSF to the DRG for a drug to reach its effect site represents a barrier for spinally administered drugs.

CURRENT SPINAL AGENTS

In this section, we will review families of agents that have been employed in humans for the management of spinal nociceptive processing. In each case we will consider the preclinical and clinical work with intrathecal delivery, mechanisms, typical molecules, adverse events with an emphasis on the work considering local drug toxicity and finally future areas of target and drug development.

Opioids

Spinal Opioids

Intrathecal delivery of opioids in amphibia and in mammals (from mouse to primate) [16, 17] reliably attenuate the response to noxious somatic and visceral stimuli that otherwise evoke an organized escape behavior with minimal effects upon light touch or proprioception [18].

Mechanisms of Action

Agonist action at the mu opioid receptor leads to hyperpolarization of neuronal membranes through activation of inwardly rectifying K+ channels and depression of excitatory neurotransmitter release from the presynaptic nerve terminal by blocking the opening of voltage sensitive Ca++ channels (VSCC), both of which serve to inhibit nociceptor neuronal activation [19]. Receptor autoradiography and histochemistry has shown that mu binding and protein are limited to the substantia gelatinosa, the region in which small afferents show their principal termination, and to small, TRPPV1 (+) dorsal root ganglion cells [20, 21]. The presynaptic action blocking the opening of VSCCs leads to an inhibition of transmitter release from the nociceptive afferents and suppression of excitability in the second order neurons [17, 18]. Local delivery of opiates (delivered systemically in spinal transected animals, or by intrathecal, topical or iontophoretic methods) in the spinal cord selectively depresses discharge of spinal dorsal horn neurons activated by small (C high threshold) but not large (Aß low threshold) afferents [22-25] and blocks release of substance P from C polymodal nociceptors [26, 27]. At the spinal level μ opioids depress the bladder reflex arc, accounting for urinary retention [28]. Other effects such as sedation or depression of the CO2 response curve accounting for respiratory depression are mediated by a supraspinal action [29, 30]. Chronic spinal infusion of opiates in animal models shows clear loss of effect with continued exposure (*e.g.*, tolerance) [31, 32], although the magnitude of this tolerance effect with continuous spinal infusion in man has been debated [33-36].

Drug Molecules

While morphine is the only FDA-approved drug for intrathecal administration, other opioids have been used for chronic intrathecal infusion including hydromorphone, methadone, and fentanyl/sufentanil [37]. With respect to neuraxial administration, the hydrophilic nature of morphine makes it a particularly useful drug for this route of administration as systemic uptake and thus potential side effects are delayed. As a comparison for these opioids, octanol/water partition co-efficients(cLogP) are morphine: 0.9,hydromorphone: 1.6; Fentanyl: 3.8; Methadone; 4.0; Sufentanil: 4.2. In general higher cLogP values result in more rapid clearance from the intrathecal space (by meningeal and vascular uptake) and correspondingly shorter durations of action [38-40]. The hydrophilic nature of morphine accounts in part for its long residence time in the spinal space and for a comparatively long duration of action thereby making bolus drug delivery a useful means for intrathecal delivery.

Adverse Events

Following intrathecal opiates, clinically significant sedation, altered mental status, nausea/vomiting, respiratory depression [41, 42] and these end points are considered to represent supraspinal mu opioid receptor activation [43]. Urinary retention is mediated by a spinal effect upon sacral mu receptors (*e.g.*, produce by mu opiates and reversed by naloxone) of the bladder–spinal bladder reflex secondary to a suppression of small afferent input and increased external sphincter tone [44-46]. Pruritus can be mediated by a peripheral, nonopioid receptor mediated effect on mast cells degranulation (*e.g.*, not naloxone reversible and with a minimal effect of agents such as fentanyl and sufentanil in

contrast to morphine and hydromorphone [47] and by an action upon mu receptors in a hypothesized brain stem site [48]. From a toxicological perspective, preclinical large animal safety studies have shown no significant effect upon spinal morphology, but has revealed the formation of intrathecal granulomas (*e.g.*, an aseptic space-occupying mass proximal to the catheter tip composed of inflammatory cells that have migrated from the meninges) to be associated with the intrathecal infusion of high concentrations of morphine, hydromorphone and methadone, but not alfentanil or fentanyl) [49-55] but see [56]. Preclinical work has shown that in accord with the lack of an opiate structure activity relationship (*e.g.*, granuloma inducing potency: morphine > fentanyl), the granuloma formation was not prevented by concurrent opiate receptor antagonism [54]. Preclinical work has pointed to the potential role of meningeal mast cell degranulation in granuloma formation [54]. In humans, comparable intrathecal masses have been observed after continuous infusion of high concentrations of most opioids including morphine and hydromorphone, but less so with fentanyl [53, 57].

Future Directions for Spinal Opiates

The pharmacodynamics of mu receptors has proven to be complex and suggest several promising avenues for future development: i) Mu opioid receptors occur as splice variants. Efforts have been made to develop ligands targeted at these splice variants [58, 59]. ii) Mu receptors couple through Gi/o protein in a ß arrestin dependent fashion such that ligand occupancy yields receptor internalization and desensitization of G protein signaling [60, 61]. Activation of mu receptors can be achieved separately from ß arrestin signaling. Such "G protein biased" ligands are believed to yield increased analgesic efficacy compared to other G protein-coupled receptors [62]. iv) Current work suggests that many G protein coupled receptors may form homo- and heterodimers [63]. Bivalent ligands interacting with these hetero-dimers, such as mu and delta receptors, have demonstrated enhanced cellular responses [64]. Thus, a mu agonist and a delta antagonist ligand display enhanced efficacy and a reduced propensity for tolerance [65]. Heterodimers constructed from opioid and nonopioid binding sites, such as bivalent Mu - CB1 (cannabinoid) [66] or μ-mGluR5 (metabotrophic glutamate receptors) [67, 68], have potent antihyperalgesic effects in avariety of models. v) Agents with delta opioid preference (*e.g.*, the effects of which are antagonized by naloxone and naltrendole) have been shown to have a spinal analgesic action in preclinical models when given intrathecally as a bolus [69-74]; or an infusion [75, 76]. This work is consistent with the presence of delta opioid receptors presynaptic on subpopulations of small primary afferents which regulate the activity of thermal and/or mechanical nociceptor [77-79] and block of release of small spinal afferent peptides [26, 69, 80-82]. One agent administered intrathecally, DADL (d-ala2-d-leu5 enkephalin), was shown to have efficacy in humans [83, 84]. These agents have been shown to not display cross-tolerance to mu opiates [75, 76]. vi) As noted above, one current hypothesis regarding granuloma formation is that it may relate to a concentration dependent degranulation of mast cells that is not mediated by an opiate receptor [54]. This dissociation suggests the possibility that agents that do not result in mast cell degranulation at concentration necessary for opiate receptor activation and have intrathecal kinetics similar to morphine might provide an alternative approach to defining an alternative to the currently available opiates. Potential molecules might for example be potent mu or delta opioid peptides [85, 86].

Alpha 2 Agonists

Spinal Alpha 2 Agonists

Alpha 2(α_2) agonists administered intrathecally have been shown to have efficacy in a variety of animal pain models including those associated with acute tissue injury and neuropathic pain states [87, 88].

Mechanisms of Action

Three relevant subclasses of $\alpha_2$agonists, α_{2A}, α_{2B}, α_{2C}, have been shown [89]. *In situ* studies have shown that subtype messages are present in sensory neurons: α_{2C}, > α_{2A}, >> α_{2B}, whereas in spinal dorsal horn the message levels are: α_{2B}> α_{2c}> α_{2A} [90]. Interestingly, discrepancies are noted between the α_2 subtypes as defined by message and immuno-histochemistry (see [91]). Spinal $\alpha_2$adrenergic receptors display several characteristics: i) binding is present presynaptically on C-fibers and postsynaptically on spinal neurons; ii) agonists are believed to alter spinal nociceptive processing by preventing opening of voltage-sensitive Ca channels (blocking release) [92, 93] and increasing potassium conductance leading to hyperpolarization of dorsal horn neurons through increased pK conductance [87, 88, 94, 95]. Consistent with their pharmacology, the analgesic activity is reversed by $\alpha_2$antagonists such as atipamezole [96-98]. At the spinal level, α_2 agonists reduce preganglionic sympathetic outflow, accounting in part for their hypotensive actions, while sedation is mediated supraspinally [99]. A similar mechanism of action to opioids is noted, albeit through distinctly different receptors that are often co-localized. Tolerance with repeated exposure has been reported, but cross-tolerance with mu opioids does not occur [100, 101].

Drug Molecules

Dexmedetomidine, clonidine, and xylazine are α_2 agonists used systemically in human and veterinary clinical practice, however, clonidine is the only $\alpha_2$agonistwith FDA approval for spinal (epidural) delivery in humans. Perhaps not surprisingly, the addition of clonidine to neuraxial morphine enhances analgesic efficacy [102, 103].

Adverse Events

The use of α_2 agonists such as clonidine is largely limited by sedation, systemic hypotension, and bradycardia that are likely produced by a central sympatholytic effect and activation of α_{2A} in the medulla. Chronic epidural [104] and limited intrathecal large animal [49] preclinical safety work has shown no evidenceof spinal toxicity.

Future Directions for Spinal Alpha 2 Agonist

While some work has suggested that analgesia and sedation may be separable if different sub-classes of α_2 receptors can be targeted [98, 105, 106], such differentiation

is not supported by studies in gene-altered mice [107], and the work has not led to definitively distinguishable agents. The cLogP of clonidine (2.6) and dexmedetomidine (3.0) reflect upon the short duration of action and rapid systemic exposure that occurs with these spinal agents suggesting that a more polar $\alpha_2$agonist might have virtue.

Spinal Sodium Channel Blockers

Spinal Antagonists

The original drugs employed for spinally directed therapy were cocaine and derivatives [108, 109]. Those incredibly insightful studies performed over 100 years ago defined the potent anesthetic and motor block produced by intrathecal cocaine and the residual presence of analgesia to pain and temperature [110]. Preclinical studies have typically shown minimal effects upon acute nociception at sub-motor impairing doses of conventional sodium channel blockers given intrathecally. However, they are reported to reliably result in a remarkable analgesic synergy with intrathecal agents such as opiates or alpha 2 agonists with minimal attendant effects upon motor function in animal models [111-114] and in humans [115-117]. There is a clinical literature that suggests that neuraxial local anesthetics may display a tachyphylaxis with chronic exposure that may respond to different local anesthetics [118]. However, systematic clinical evidence of this impression is controversial (see [119]. Nerve block studies do not display such changes, suggesting that if tachyphylaxis does occur it reflects some system level change [120].

Mechanisms of Action

The common mechanism of all clinically employed "local anesthetics" is to block the voltage-gated sodium channel (Na_v). Na_vs consist of an α-subunit complex that forms the transmembrane spanning pore and auxiliary β-subunits [121]. The channel expresses molecular components mediating the voltage sensing and gating elements that result in the voltage-dependent opening and voltage-independent closing of the channel. Importantly, the typical anesthetic with pKa >7.4 is unionized at extracellular physiological pHs, penetrates the membrane, reionizes in the more acidic intracellular environment and in the ionized (protonated) state blocks the channel from the intracellular side. Several general properties of the block are appreciated. The differential block of motor axons and low vs. high threshold sensory afferents reflect the greater sensitivity of small vs. large afferents to block of the conducted action potential. As local anesthetics bind to sodium channels in an activated state, the initiation of blockade is facilitated in neurons discharging at a high frequency (*e.g.* a state-dependent block [122-124]. Previous speculations [112] have pointed to the role of impedance mismatching at the extensive afferent branch points within the dorsal horn. This would lead to an increased probability of conduction failure in the small (C-fiber) afferents [125] and an increased susceptibility of the terminal depolarization to be blocked by low concentrations of local anesthetics.

Nine isoforms have been identified with distinguishable activation properties and tissue distributions [126]. Of particular note, $Na_v1.4$ and $Na_v1.5$ are present in skeletal and cardiac myocytes. $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ are predominantly expressed in small sensory DRGs/afferents, while NaV 1.1 and 1.6 are found more highly expressed in large DRG/axons [127]. Clinically employed local anesthetics (amide and ester) are essentially non-selective in their blocking of different sodium channels (see [124]). Several sodium channel isoforms are sensitive to the puffer fish toxin, tetrodotoxin (TTX) ($Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.4$, $Na_v1.6$, and $Na_v1.7$), while others ($Na_v1.5$, $Na_v1.8$, and $Na_v1.9$) are resistant to TTX [124, 128]. Importantly, following chronic inflammation and peripheral nerve injury, prominent increases in the expression of small afferent Na_vs has been noted and such increases appear to be associated with the initiation of ongoing (ectopic) afferent traffic [129]. The use of antisense, siRNA and murine mutations have indicated that impairment of $Na_v1.3$, 1.7,1.8 and 1.9 expression has varying antihyperpathic effects in models of inflammatory and neuropathic pain states [127, 130, 131]. Specific gain and loss of function mutations in the $Na_v1.7$ channels have been identified in humans wherein there is an increased and decreased pain state, respectively [132, 133].

Drug Molecules

Avariety of local anesthetics are employed with two common structural motifs: Hydrophobic (aromatic residue) and hydrophilic (tertiary or secondary amines) domains separated by an ester procaine, 2-chlorprocaine, tetracaine) or amide (lidocaine, bupivacaine, ropivacaine) linkage. The principal differences between drugs being use dependency, effective concentrations and duration of action. Though differences in relative potency with respect to blocking different Na_v subtypes have been reported, such selectivity remains controversial and in part reflect variation in on and off rates of binding and channel opening and inactivation properties rather than a specific difference in Na_v affinity [123, 124]. The properties of local anesthetic actions at the channel have been extensively reviewed elsewhere [134, 135].

Adverse Events

Adverse events such as motor weakness and anesthesia after intrathecal delivery of currently employed local anesthetics reflect side effects related to the block of the several voltage-gated channels associated with large sensory and motor axons and spread of the block to higher centers (*e.g.* "total spinals") and to non-neuraxial tissues such as cardiac myocytes. As noted, the large axons are relatively resistant to conduction block. Neurological signs secondary to intrathecal local anesthetics have been identified in patients with uncomplicated spinal anesthesia wherein distal lower extremity pain was reported. Transient neurologic symptoms were reported with relative risk being higher for lidocaine compared to bupivacaine, prilocaine, procaine and mepivacaine [136]. Preclinical safety evaluations with intrathecal local anesthetics such as lidocaine, bupivacaine and ropivacaine have been marked initially by mitochondrial vacuolization, mild focal edema, with evidence of change in the lamellar structure of fibers and Schwann cells in rat and dog models [137, 138]. The molecular mechanisms of the

local anesthetic toxicity are not understood. Work with intrathecal TTX has shown that long-lasting sodium channel block itself is not associated with a specific toxicity [139]. Small local anesthetic molecules can have effects on lipid membrane components, which reflect the detergent nature of these amphiphillic molecules [140]. Though the mechanisms are not understood, the afferent toxicity does appear to be associated with increased intracellular calcium in the DRG [141].

Future Directions for Spinal Sodium Channel Blockers

The current advances in our understanding of the Na_v subtypes and their differential distribution in the body and particularly in the neuraxis, as well as the upregulation in their expression after injury leading to ectopic activity and increased neuronal excitability, offers evident opportunities for creating drugs that target those elements that might be most altered by the pain states. Several strategies have been employed. As reviewed elsewhere, there has been a great deal of effort to define structures that prefer one channel over another [142, 143]. Preclinical work with intrathecally-delivered molecules targeted at $Na_v1.7$ [144] and 1.8 [145] has shown preclinical efficacy. While much attention has been paid to the TTX-resistant sodium channels in sensory neurons (*e.g.*, $Na_v1.8$ and 1.9) an important role for TTX sensitive channels cannot be excluded. Intrathecal TTX has been reported to have mild effects upon acute and inflammatory nociceptive end points [146]. $Na_v1.3$ and 1.7 produce TTX-sensitive sodium currents and have been shown to contribute to ectopic firing in injured neurons. As noted above, $Na_v1.3$ expression increases with nerve injury. Intrathecal knockdown of these channels with antisense reduced their upregulation in nerve injury and reduced the behaviorally defined tactile allodynia [147-149]. The use of intrathecally-delivered siRNA and antisense targeting these structures clearly offers a direct approach to targeting these membrane proteins [127, 130, 131]. An alternate strategy for selective targeting has been the use of non-selective blockers that are restricted in terms of the cells to which they have access. The quaternary lidocaine molecule (QX314) cannot readily penetrate the cell. However, activating membrane channels such as the TRV1 channel has been reported to allow QX314 entry into the neurons and to block the Na_v channel from the interior and result in a selective block of the TRPV1 expressing primary afferent [150]. Intrathecal QX316 has been reported to produce significant pain behavior after intrathecal delivery in rodent models and this may reflect upon its ability to concurrently activate the TRPV1 channel [151]. It should be noted that while this section considers the role of sodium channel blockage in reducing axon excitability, it has been shown that conditions leading to an enhanced expression of sodium channels often lead to a reduction in the expression of potassium channels which further contributes to membrane excitability [152]. Increasing potassium conductance with potassium channel agonists or increasing the expression of potassium channels can serve to move the membrane towards a hyperpolarized state and normalize (reduce) otherwise enhanced axon, DRG and terminal excitability, an action associated with an antihyperalgesic action [153, 154].

Spinal Calcium Channel Blockers

Spinal Antagonists

Ziconotide (Prialt), discovered in the venom of fish-killing marine snails [155], was demonstrated to be a potent antihyperpathic agent in rodents and humans after intrathecal delivery as a bolus or an infusion [156-159]. Ziconotide was observed to be a highly selective ligand blocking the N-type voltage-sensitive calcium channel (VSCC) [160].

Mechanisms of Action

VSCCs facilitate neurochemical cell signaling through mobilization of presynaptic vesicles for neurotransmitter release. The N-type calcium channel is most important for neurotransmission in sensory neurons. This calcium channel subtype is densely expressed in presynaptic nerve terminals in the spinal cord superficial dorsal horn and dorsal root ganglia. In the presence of nerve injury these channels are upregulated [161] and blocking these channels has shown to produce antinociceptive effects [162] that do not show tachyphylaxis (tolerance) [163].

Drug Molecules

Ziconotide, an ω-conotoxin derived from the cone snail, is a calcium channel blocker that binds with high affinity and physically blocks the N-type calcium channel. It is a stable, water soluble, large 25 amino acid, polybasic peptide containing three disulfide bridges with a *molecular weight* of 2639 Da. Ziconotide is FDA approved for intrathecal use for chronic, severe pain.

Adverse Events

In animals, ziconotide produces dose-dependent body shaking and ataxia [157, 164, 165]. A narrow therapeutic index reflecting nonspinally mediated side effects such as dizziness, nausea and somnolence has limited the human clinical utility of ziconotide. Extensive large animal preclinical safety evaluations have emphasized the lack of tissue toxicity of this molecule at clinically useful concentrations [166].

Future Directions for Spinal Calcium Channel Blockers

i) Currently the only N type channel blocker approved as a therapeutic is Ziconotide. Considerable work has focused on the development of other conopeptides as well as small molecules [160, 167]. Alternatively, there is considerable interest in altering N-type VSCC function by impeding its membrane trafficking and such strategies have been shown to alter nociceptive processing [168]; ii) VSCCs are broadly divided into two types: high-voltage-activated (HVA) and low-voltage-activated (LVA) channels. HVA channels are further divided into L-, P/Q-, N- and R-type channels. LVA channel include the T-type channel. Each channel locates in a distinct area in the peripheral nerve and CNS and contributes in different ways to nociceptive transmission (see [169]). Spinal blockade of R- and T-type but not L and P/Q type VSCCs attenuated formalin-induced pain behavior [170-172]. In other work, intrathecal PQ-preferring toxins displayed efficacy in neuropathic pain models [173]. Intrathecal delivery of T-type calcium channel blockers

(ethosuximide and mibefradil)produce analgesia in rodents [171, 174]. iii) An alternate therapeutic target thought to involve regulation of VSCCs is the α_2 delta calcium channel auxiliary subunit. This subunit is the binding site for gabapentinoid drugs in the dorsal horn. In preclinical models, gabapentin and pregabelin have been shown to have potent effect in a variety ofhyperalgesic pain [175-178] Preclinical results indicate adequate spinal pK with a very hydrophilic cLogP (-1.13). In a recent study, however, it was unexpectedly reported that intrathecal infusion of gabapentin failed to alter pain assessments in human patients suffering from a variety of neuropathic and non-neuropathic pain states [179].

NMDA Ionophores

Spinal NMDA Antagonists

A wide variety of NMDA ionophore antagonists given intrathecally have been reliably shown to have potent attenuating effects upon the hyperpathia resulting from a variety of tissue [99, 180] and nerve injury [181] facilitated pain states in animals and, though not approved, in humans [182].

Mechanisms of Action

The NMDA receptors are heteromeric protein complexes assembled from three families of NMDA receptor subunits (NR1, NR2 and NR3) each with multiple distinct subunits [181]. When activated it allows the influx of sodium, potassium and calcium. Glutamate, released from afferents and interneurons in response to high threshold noxious input from C fibers, binds target sites on the NMDA receptor. These receptors are densely expressed in spinal cord dorsal horn on the terminals of the primary afferent, on second order neurons as well as in non-neural cells such as astrocytes and oligodendroglia. Activation of the channel requires the presence of occupied binding sites on the ionophore for glycine and polyamines [183]. At resting membrane potentials a Mg++ ion provides a block in the channel that effectively prevents calcium flux through the channel. In the face of repetitive activation, the membrane displays progressive depolarization, relieves the Mg++ block and allows calcium flux [183], leading to a cascade initially described electro-physiologically, as wind-up [184]. Blocking NMDARs inhibits the wind-up phenomenon of spinal dorsal horn neurons [185, 186]. This increased excitability further results in activation of a variety of protein kinases (Mitogen activated kinases and PKA and PKC) which, respectively, increases protein synthesis, phosphorylates channel and enzymes that lower their thresholds for activation and enhances their ion permeability leading to hyperalgesic states [187-190].

Drug Molecules

Numerous NMDA antagonists have been employed for preclinical work showing the antihyperpathic effects of intrathecal NMDA antagonists [191-194]. These include channel blockers such as ketamine (cLogP: 3.1), MK-801 (cLogP: 2.7) and memantine (cLogP 2.0), and competitive antagonists such as 2 amino 5 phosphonovalorate (cLogP=-2.32), glycine site blockers such as 7 Chlorothiokynurenic acid (cLogP: 2.7). No NMDA antagonists have been approved for human neuraxial use.

Adverse Events

Single intrathecal injections of ketamine did not produce histological alterations in the dog [195] but resulted in spinal cell death in the neonatal rat [196]. After repeated intrathecal delivery in rabbits [197] or continuous infusion in dogs [198], Ketamine and a number of other NMDA channel-blocking antagonists including MK-801, dextrorphan, dextromethorphan and memantine and the nonopiate agent D-methadone, and to a lesser degree the glutamate antagonist 2AP5,resulted in prominent spinal pathology reflecting parenchymal necrosis in dogs and/or sheep [198, 199]. These spinal effects, where examined, appear to be concentration-dependent and, with the exception of the neonatal model, the therapeutic ratio (*e.g.*, therapeutic dose/minimum toxicity dose-concentration) has not been defined. It should be noted that a weakness of these studies is that there was no corresponding assessment of the efficacy of the intrathecal infusion was assessed relative to the dose required to produce pathology in these animals models (but see [196]).

Future Directions for Spinal NMDA Antagonists

i) Preclinical work and anecdotal clinical data clearly indicate the efficacy of spinal NMDA antagonism. However, in light of the controversy related to the intrathecal toxicity of NMDA antagonists (see above), characterization of the intrathecal therapeutic ratio for the NMDA antagonist is necessary to define the relative safety of these agents. While their ability to produce untoward histopathology is evident, it is not clear how close the toxicity is in the adult, continuously infused animal to the dose-producing beneficial consequences. ii) The NMDA ionophore is constituted of a variety of subunits. Ionophores constituted of different subunits have different binding profiles and are differentially distributed [200, 201]. One such subtype is the NR2B subunit. Specific antagonists such as Ifenprodil [202], Ro 25-6981 [202, 203] and Conantokin G [204] has antihyperpathic effects in models of spinal injury and facilitated processing. Other NMDA ionophore-associated targets for which ligands have been shown to produce analgesia include the glycine binding site and the polyamine binding site [201, 205]; iii) Magnesium sulphate is a noncompetitive antagonist of the *N*-methyl-d-aspartate (NMDA) receptor and thus can modify nociceptive modulation [206-207]. Limited preclinical safety studies have been performed examine the effects of bolus delivery in several species. At least one preclinical paper has reported evidence of neurotoxicity after intrathecal delivery [208]. Chronic spinal exposures have not been examined. A variety of reports have noted activity in humans after intrathecal and epidural delivery [209]. iv) Agmatine is an endogenous neuromodulator present in the brain and spinal cord that has both NMDA receptor antagonist and nitric oxide synthase inhibitor activities. It has been reported to have antihyperalgesic properties after systemic and intrathecal delivery [210]. v) The primary focus of glutamate ionotrophic receptors has been on the NMDA. However, the AMPA receptor plays a prominent role in acute dorsal horn evoked excitation as a sodium selective ionophore [211].

Agents such as Tezampanil (*LY-293558, NGX-424)* given spinally in preclinical studies has effect upon postoperative pain and spasticity [212, 213] and its action were associated with changes in motor function, a fact consistent with the prominent role of AMPA in motor neuron excitation. In humans oral dosing has indicated some effects upon afferent evoked hyperalgesia [214]. vi) The AMPA receptor is composed of GluR1-GluR4 subunits. In the face of injury, there is a change in subunit trafficking and ionophore composition such that the ionophore becomes calcium permeable. Intrathecal antagonists selective for the calcium permeable AMPA site, such as joro spider toxin, reduces the development of secondary mechanical allodynia evoked in tissue injury models [215-216].

GABA-A

Spinal GABA-A Activation

Preclinically, intrathecal GABA-A agonists such as muscimol or isoguavacine have been reported to display efficacy in neuropathic pain models [217-221]. Intrathecal benzodiazepines and neurosteroids have been shown precliincally in rodent models to attenuate tissue and nerve injury hyperpathic states [218, 222-226]. In dogs, intrathecal benzodiazepines have been shown to depress nociceptive reflexes [227]. In humans, bolus intrathecal Midazolam was reported to display efficacy after bolus deliveryin postoperative, low back and labor pain [228-231].

Mechanisms of Action

The GABA-A receptor is a GABA gated chloride ionophore composed of five subunits, each with four transmembrane spanning domains. With multiple subunits, not surprisingly, several different pharmacological and functional phenotypes of the receptor have been identified [232]. GABA binds at an α and a β subunit [233] to activate the Cl conductance. Importantly, numerous agents interact with specific sites on the GABA-A ionophore to serve as positive allosteric modulators. Thus, benzodiazepines (*e.g.*, midazolam, diazepam) bind to specific populations of GABA-A ionophores that possess both an α and a γ subunit. This binding forces a conformation where GABA displays a higher affinity increasing the frequency of opening of the ion channel [234]. Similarly, several neurosteroids such as allopregnanolone will bind to the GABA-A ionophore and force conformational changes enhancing GABA effects [235, 236]. Immunohistochemical studies have shown dense staining in spinal Rexed laminae for several alpha, beta and gamma subunits likely corresponding to primary afferents [237, 238] presumably localized on primary afferent terminals. Electrophysiological studies have indeed shown GABA-A receptors to be present on both large and small afferents [239] and such studies have emphasized the potent regulation by benzodiazepines of the excitability of small, nociceptive, primary afferents through their associated GABA-A receptors [240]. There is not enough space here to review the differential distribution of functionally distinct GABA-A receptor phenotypes, but electrophysiological and knockout studies have shown considerable phenotype diversity in the neuraxis [241, 242].

Drug Molecules

As noted above, the common agonists employed in pharmacological studies of the GABA-A receptor are muscimol (cLogP = -0.7). Agents serving as allosteric modulators include benzodiazepines (midazolam; cLogP = 3.16) and neurosteroids (allopregnanolone; cLogP= 3.7).

Adverse Events

Activation of nonspinal GABA-A ionophores has prominent sedative, anxiolytic and amnestic effects. At the spinal level enhanced GABA-Aactivity has been shown to have effects upon motor function [243]. The use of intrathecal midazolam in man has been reported as noted above. However, there has been concern about the potential for spinal toxicity [243, 244]. While several systematic assessments of toxicity have shown distinct deleterious reactions [245, 246], others have failed to observed toxicity [56]. The origin of the preclinical toxicity is not known, but the acidic pH required by the formulation of midazolam may be a contributing issue (see for review [244]. In our opinion, concern over its potential for toxicity remains. Barbiturates act through the GABA A ionophore, but their spinal action may be associated with a cauda equine syndrome. Such has been the case after inadvertent intrathecal injection of sodium thiopental [247].

Future Directions for Spinal GABA-A Agonists

As noted above, a high degree of heterogeneity of the GABA-A receptor structure/subtype has been observed, which raises the possibility of defining specific structures targeting these subtypes [242, 248-250]. Activation of spinal GABA-A ionophores either directly through agonist activation or by positive allosteric modulators has significant preclinical support for further development as reviewed above with benzodiazepines and neurosteroids. Interestingly agent such as Etifoxine may act by stimulating allopregnanolone synthesis in the spinal cord [251]. Current work has shown that subtype specificity may be achieved and that they may have different effects upon neuronal inhibition in different systems [252].

GABA-B Receptors

Spinal GABA-B Agonists

Baclofen, the clinically approved GABA-B agonist, has potent stereospecific suppressive effects upon motor tone [253] and is widely used clinically to regulate motor tone in the case of spasticity [254-256]; but it has also been implicated in having suppressive effects upon nociceptive processing after intrathecal delivery in preclinical models of facilitated processing [217] and hyperpathia in mono and polyneuropathies and spinal injury [220-221, 257-259] and in humans [260, 261].

Mechanisms of Action

The GABA-B receptor is a metabotrophic receptor coupled to intracellular signaling through Gi and Go proteins that, when stimulated, activate K+ and inhibit Ca2+ channels *via* the βγ complex, with the α subunit regulating adenylate

cyclase [262]. They are dense in laminae I and II in the dorsal horn on small afferent terminals and on 2nd order neurons [263]. DRG neurons of various diameters also express GABA-B immunoreactivity. At the electron microscopic level, the immunoreactivity is present on myelinated and unmyelinated fibers and on the dendrites of dorsal horn neurons. Electrophysiological studies show that GABA-B agonists initiate hyperpolarization through an increase in K conductance through G protein coupling and attenuation of evoked primary afferent transmitter release through block of opening of voltage gated Ca channels. GABA-B receptors are present on motor neurons and serve to reduce their excitability leading to flaccidity at doses that are similar to those yielding antinociception.

Drug Molecules

The common GABA-B agonist is baclofen (cLogP = 1.3), which exerts its effects in a stereospecific fashion [253, 264]. GABA-B selective antagonists, such as saclofen [265], block the effects of these agents.

Adverse Events

Intrathecal baclofen produces a prominent dose-dependent motor weakness in animal models and humans [253]. In humans, this effect has a particular therapeutic benefit in managing clinically relevant spasticity (see [256]). Intrathecal baclofen has undergone one published systematic toxicology evaluation in dogs involving a 28-day continuous infusion without evidence of toxicity [55, 266]. Intrathecal baclofen has been reported to result in intrathecal space-occupying masses, but this has proven to be controversial [267, 268].

Future Directions for GABA-B Receptor Targeting

Other GABA-B agonists have been identified and include agents such asCGP35024 and the more potent CGP 44532, which were reported to induce antinociceptive responses at doses below those that cause sedation [257, 269]. It has become clear that regulation of GABA-B activity is associated with a role for positive allosteric modulators to potentially reduce unwanted drug effects. These agents interact with discrete domains of the GABA-B receptor stabilizing it in the active state involved in G-protein coupling. Such agents have been found to modulate the GABA-B receptor analgesic activity [270].

Spinal Cholinesterase Inhibitors

Spinal Cholinesterase Inhibitors

Neostigmine is a cholinesterase inhibitor that yields antinociceptive effects in a variety of preclinical models [106, 271-275] in several species such as rodents and sheep [276]. Bolus intrathecal delivery of neostigmine has been reported in humans to have significant effects upon postoperative pain [277-279].

Mechanisms

Intrathecal cholinesterase inhibitors are considered to increase the local extracellular levels of acetylcholine. Spinal delivery of a variety of cholinergic antagonists has indicated the importance of muscarinic receptors and in particular several M subtypes in mediating these antinociceptive actions [280]. These findings are consistent with the antinociceptive effects of intrathecal muscarinic agonists [281]. Spinal muscarinic receptors in the spinal cord are strongly expressed in the superficial dorsal horn [282].There are five subtypes (M1-5) [283, 284] divided into two broad groups: M1/M3/M5 coupled to $G_{q/11}$ proteins, activating phospholipase C - IP3—diacylglycerol (DAG) cascade yielding increased Ca^{2+} and activation of protein kinase C and mitogen-activated protein kinases. M2 and M4 receptors (M2-class) coupling to $G_{i/o}$ proteins and inhibiting opening of voltage-gated calcium channels [284]. M1/3/5 receptors induce membrane depolarization by enhancing cation currents and inhibiting potassium channels [285, 286], while M2 and M4 channels activate potassium channels and inhibit some voltage-gated Ca channels (especially $Ca_{v2.2}$), leading to hyperpolarization and block of transmitter release [287]. M_2 and M_4 knockout animals show a robust attenuation of the analgesic effects produced by muscarinic agonists [165, 288, 289] Interestingly, stimulation of M_2 and M_4 receptors inhibit spinal glycinergic function which might lead to a paradoxical hyperpathia, whereas stimulation of the M3 activation potentiates synaptic glycine release [290]. The events underlying the effects upon spinal nociceptive processing have been proposed to reflect several mechanisms.1) effect of muscarinic receptor subtypes in reducing afferent terminal excitability (reducing glutamate release) [289]; and, 2) activation of muscarinic receptors on glycinergic and GABAergic interneurons enhancing dorsal horn inhibitory amino acid release through muscarinic subtypes, such as the M2 and M4 receptors [290, 291].

Adverse Events

The safety of intrathecal neostigmine has been examined systematically in rats after multiple bolus deliveries and after chronic infusion in the dog [292] and no apparent tissue toxicity was noted. In humans, intrathecal neostigmine has been shown to provoke nausea and vomiting [277].

Future Directions

Considerable work has focused on the development of novel muscarinic agonists and cholinesterase inhibitors [293]. As noted above, there is considerable evidence that subtypes of muscarinic receptors may be involved in the spinal antinociceptive actions and anticholinesterase targeted agents.

Adenosine

Intrathecal Adenosine

Delivery of adenosine and associated ligands can have potent antihyperalgesic effects in models of acute nociceptive processing [294], neuropathy [294-298] and inflammatory pain [299]. In humans, intrathecal adenosine has been shown to reduce allodynia in experimental pain models [300, 301] and in patients with neuropathic pain [302,303]. Negative results with intrathecal adenosine have also been reported [304].

Mechanisms

Adenosine can activate four G-protein-coupled receptors: A_1, A_{2A}, A_{2B}, A_3 [305]. A_1receptors are expressed on small-to-medium-diameter neurons of the DRG and on dorsal horn neurons [306-309] and serve to inhibit synaptic transmission through presynaptic inhibition of neurotransmitter release and on postsynaptic inhibition of excitatory transmission [309, 310].

Adverse Events

In preclinical studies, A_1, agonists and A_{2A} antagonists reversibly depressed the volume-evoked micturition reflex [311, 312] and produced motor weakness. An unexpected observation was that chronic exposure of spinal systems to A_1agonists led to a hyperalgesic state [313]. The safety of intrathecal adenosine has been examined systematically in rats after multiple bolus deliveries and after chronic infusion in the dog [300, 314] with no adverse signs or histopathology. Studies with intrathecal delivery of the A_1 agonistR-phenylisopropyl adenosine (R-PIA) also failed to show histopathology [315].

Future Directions

It has been reported that A_{2A}receptor agonists may produce a long-term reversal of allodynia in mononeuropathies [316]. It has been proposed that the mechanisms of this long-term effect may reflect the effects ofA_{2A}agonists as potential glial inhibitors. The complexity of this possible drug effect is also reflected by the observation that A_{2A}receptors may serve to stimulate glutamate release and that A_{2A}antagonists may have protective effects by reducing that excitatory effect [317].

Cyclooxygenase Inhibitors

Cyclooxygenase Inhibitors

Considerable work has shown that in the face of persistent small afferent input there is an increase in the release of cyclooxygenase products such as PGE2 [318-320]. This increase results from activity in constitutively expressedCOX-2 in spinal dorsal horn neurons and glia [321]. The role of these PGs in the development of a sensitized dorsal horn state of processing is mediated by several mechanisms including enhanced opening of voltage-gated calcium channels [322] and the loss of intrinsic inhibition by an inhibitory effect upon dorsal horn glycine receptor function [323]. Intrathecal delivery of COX-1/2 or COX-2 selective inhibitors has been shown to have antihyperalgesic effects in a variety of models producing central sensitization [318, 324-326]. Spinal COX-1 inhibition has attenuating effects upon postoperative pain [327] and Ketorolac is a mixed COX-1/2 inhibitor. Preclinical work has shown no evidence of toxicity in repeated bolus injections in the rat and continuous intrathecal infusion in the dog [328, 329] and was well tolerated in humans [330]. In human studies, intrathecal ketorolac delivered as a bolus had modest effects in human experimental pain models [331], enhanced morphine analgesia after total knee replacement [332], but had no effects on postoperative pain [333] or hip arthroplasty [334].

Other Potential Pharmacological Spinal Targets

In this section, we will briefly consider the role of several pharmacological targets that have been shown to play a role in spinal nociceptive processing. It should be stressed that virtually all of the mechanistic studies defining pain processing have shown the importance of spinal systems. Accordingly, future pharmaceutical targets may likely arise from one or more of systems that have been shown to have actions on the behaviorally defined pain end point after intrathecal delivery in one of more preclinical models (see [10-11]). Several of these will be summarized below. Importantly, most of these agents and their associated targets have not undergone any systematic assessments of toxicity and may carry with them unexpected/off-target membrane effects.

Metabotrophic Glutamate Receptors (mGluR)

Eight mGluRs (mGluR1-8) have been identified and these are divided into three groups [335]: Group I (mGluR1 and mGluR5) stimulates phospholipase C (PLC); Group II (mGluR2 and mGluR3) and Group III (mGluR4 - mGluR8) inhibit adenylate cyclase [335, 336]. In the brain and spinal cord, nGluRs have been identified on primary afferents, neurons and glia [337, 338]. Group I is localized postsynaptically, whereas Group II and Group III are present largely on the presynaptic terminal [339]. Activation of Group I mGluRs have been implicated in the processes of central sensitization and persistent nociception, whereas activation of Group II mGluRs has been shown to suppress facilitated states [340]. Accordingly, antagonists of Group I mGluRs and agonists of Groups II and III mGluRs have shown analgesic activity in neuropathic or inflammatory pain states [341-348] and accordingly represent a promising target for the development of future spinally-targeted agents [349, 350].

Toll Receptors

Toll-like receptors (TLRs) are a family of pattern recognition receptors that recognize a variety of injury-associated molecular structures. They are located on neuronal and non-neuronal cells in the spinal cord and signal through downstream pathways that often lead to anelaboration of a variety of proalgesic/inflammatory cytokines(DRG) [351, 352]. Intrathecal delivery of TLR4 antagonists has been shown to have ameliorating effects upon inflammatory and neuropathic pain states [353] and has been implicated in the phenomena of opiate-induced hyperalgesia [354]. Novel structures for blocking TLR activation through an interaction with the TLR4 ligand or targeting downstream signaling have been of therapeutic interest [355-357].

Cannabinoids

Cannabinoid receptors (CB1r and CB2r) are 7 trans-membrane, G-protein- coupled receptors that negatively couple through Gi/o proteins [358]. Spinally, CB1 receptors are expressed in neurons while CB2 receptors are expressed on microglia, where the CB1 agonists reduce excitatory transmitter release and CB2 receptors attenuate microglial activation [359, 360]. Intrathecal delivery of both CB1 and CB2 preferring ligands reduced facilitated states such as the

formalin model, hyperpathia in neuropathy models and in tumor bone pain in rodents [361-363]. As many of these ligands have very high cLogPs, appropriate formulation in spinally compatible vehicles is an important consideration in their development for spinal use.

Purine Agonist/Antagonists

Adenosine triphosphate is widely released in the nervous system [364] and has been shown to interact with the P2 receptor family, which is divided into P2X ligand-gated ionotropic receptors (further divided into 7 subtypes) and P2Y\G-protein-coupled receptors divided into 8 subtypes). The P2Y receptors may couple through either G_q/G_{11} to activate the phospholipase C/inositol triphosphate ($InsP_3$) endoplasmic reticulum Ca^{2+}-release pathway (the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors) or through to $G_{i/o}$, which inhibits adenylyl cyclase and serves to modulate ion channel function [365]. Both P2X and P2Y receptors are widely distributed in spinal dorsal root ganglia and in spinal neurons and glia [366]. A large persuasive literature has implicated these receptors as primary activators of glia, particularly microglia, leading to the spinal release of a plethora of proinflammatory proteins and cytokines relevant to initiating facilitated pain states [367-371]. Intrathecal delivery of a variety of P2X and P2Y inhibitors results in a temporary reversal of hyperpathia after nerve injury [372-375]. Studies with a variety of approaches have strongly implicated the P2X4 subtype in spinal facilitation initiated by tissue and nerve injury [376]. Intrathecal administration of a P2X4R antisense oligodeoxynucleotide prevented the increase in P2X4R protein expression and suppressed the development of mechanical allodynia [372].

Anti-Inflammatory Cytokines (IL-10)

Activation of a variety of glial signaling cascades will lead to the release of a variety of cytokines including activation of NF-κB and results in the production of proinflammatory mediators such as TNF, IL-6, and IL-1β that activate pro-algesic cascades [377]. Conversely, these cascades can lead to the release of so-called anti-inflammatory products that may bind the soluble product (IL-1ra) and cytokines such as, IL-4, IL-6, IL-10, IL-11, IL-13, TGF-β, and various soluble cytokine receptors [378], which can regulate the inflammatory cascade [379]. As an example, intrathecal delivery of IL-10 has been shown in animal models to have therapeutic efficacy in a variety of pain models [380].

Future Targeting

An exciting component of pain modulation has been the implementation of several novel approaches to modify the function of systems that process nociceptive information. A brief note on these approaches will be given below.

Targeted Toxins

This approach takes advantage of toxin delivery to specific cell systems. Several examples can be considered.

TRPV1 Receptors

These TRPV1 channels are present on the terminals of small high threshold primary afferents and when activated by ligands such as capsaicin [381] and analogues [382] will yield activation, desensitization and loss of terminals expressing the channel after spinal delivery [383]. Intrathecal resiniferatoxin, a potent TRPV1 agonist, has been shown to have a potent antihyperalgesic effect in canine persistent pain models [384] and is currently in human clinical trials (http://clinicaltrials.gov/show/NCT00804154). A further modality for targeting TRPV1- bearing neurons takes advantage of the fact that when activated TRPV1 forms a pore that is able to pass large charged molecules. Protonated local anesthetics (*e.g.*, QX314), which cannot normally enter the axon, can pass through TRPV1 channels that have been opened by TRPV1 agonists such as capsaicin and produce a selective block of sodium channel function in the TRPV1 (+) afferent axon [385].

Toxins Coupled to Agonists for G-Protein-Coupled Receptors

G-protein-coupled receptors when occupied by their agonists will undergo internalization [386]. Composite molecules composed of the ligand and a toxin will bind to the respective GPCR. Such binding leads to an internalization of the agonists and the coupled toxin into the cell expressing the particular receptors [387]. Such GPCRs thus far intensely studied include those for the neurokinin 1 for sP. These receptors are present on cells within the spinal pain pathway. The spinal neurokinin 1 receptor is located on postsynaptic second order neurons [388]. The majority of work at present has focused on the NK1-r. However, other G-protein receptors that are likely ligands are the mu receptors. As reviewed above, mu opiate receptors located in the spinal cord are primarily located in Laminae I and II on the nerve terminals of primary afferent C-fibers and interneurons in Lamina II. Agonist binding to these receptors leads to their internalization [19].

i) Saporin, a protein derived from the plant *Saponaria officinalis,* has been coupled to a variety of ligands including dermorphin, a ligand for the mu opiate receptor, and sP, the ligand for the neurokinin 1 receptor. The internalized saporin is cleaved in endosomes within the cell and serves to block ribosylation thereby preventing protein synthesis and leading to death of the targeted cells. Intrathecal sP-saporin has been shown to lead to a robust change in a variety of pain states in several species including the rodent [389] and dog [261]. Large animal safety studies have shown target engagement after intrathecal delivery showing local knockdown of spinal NK1-r protein and message [390]. Importantly, at high concentrations motor dysfunction was observed that reflected upon the presence of NK1-r in motor neurons in the dog. Intrathecal sP-Saporin is currently in human clinical trials (http://clinicaltrials.gov/show/NCT02036281).

ii) Botulinum toxin. Botulinum toxin is constructed of a heavy chain (HC) and a light chain (LC). The HC enables the uptake of the complex into the cell. Once in the cell, the complex is cleaved freeing the LC, which is the active enzyme cleaving SNARES [391]. SNAREs play an important role in mobilizing vesicles for transmitter release and in enabling movement of certain proteins to the membrane, such as the GLUA1 AMPA receptor subunits [392]. By cleaving SNAREs, transmitter release is blocked

and intracellular trafficking may be diminished. Intrathecal BoNTs have been shown to produce antihyperalgesic effects in a number of preclinical models of inflammatory and neuropathic hyperpathia [393-396]. The BoNT uptake is considered to be ubiquitous and the potent effects upon transmitter release may include inhibitory interneurons and motor neurons [397, 398]. A single human experience has emphasized the potential of producing an enhanced pain state, perhaps through block of inhibitory amino acid release [399]. An approach to achieving specificity has employed the GPCR coupling motif described above. Here sP coupled to the LC may be delivered IT and the coupled molecule uptake is restricted to cells that express the NK1 receptor [400]. Such an approach is reversible and may find utility in long-lasting but non-terminal pathophysiological pain states.

Gene-Based Approaches

Targeting specific proteins that play a role in nociceptive processing has been accomplished with several methodologies including intrathecally-delivered antisense and viral and nonviral transfection [401-403].

i) Intrathecal antisense has been employed to reduce expression of a variety of proalgesic mediators, including growth factor cellular matrix proteins (thrombospondin) [404] and receptors relevant to nociceptive processing, including the NK1 receptor for substance P [405], purines [372]; GFRα1, receptor for GDNF [406], NMDA receptors [407]; glutamate metabotrophic receptors [408-410], P2X3 [411, 412], TrpV1 receptors [413], TrpV1 receptors [413], CCR2 (canonical receptor for MCP-1) [414], synaptic scaffolding proteins [415], a variety of kinases such as P38 MAPK [416, 417], phospholipases [418], and a variety of channels including sodium ($Na_v1.8$: [419, 420] and calcium channels (T-type: [421, 422]). Inactivating transcription factors by employing an oligonucleotide decoy protein represents an alternate approach to prevent activation otherwise initiated by a pain state [423].

ii) Transfection by intrathecal delivery of viral vectors encoding a given protein or the use of nonviral methods such as nanoparticles or permeabilizing systems [403] can enhance the targeted protein expression *in vivo*. Several examples may be noted. i) Dorsal root ganglion neurons virally transduced with a vector coding for glutamic acid decarboxylase (QHGAD67) increase the release of spinal GABA to produce an analgesic effect [424-425]. ii) Animals displayed increased expression of endomorphin-2, an opioid peptide [426], or the preprohormone for enkephalin [427] or ß endorphin [428] after intrathecal vector transfection elevated thresholds. iii) The use of intrathecal viral vectors or plasmid delivery to increase appropriate antihyperalgesic targets has been shown to have utility. Thus, increased spinal expression of IL-10 (anti-inflammatory cytokine)or plasmid delivery was anti-hyperalgesic [424, 429-431]. Increasing MAPK (Mitogen activated protein kinase) phosphatase-1 reduces activation of P38 MAPKs and this resulted in reduced nerve injury-evoked increases in inflammatory cytokines and chemokines and hyperalgesia [432]. Increased expression of the Ca^{2+} channel-binding domain 3 (CBD3) peptide blocks function of Ntype calcium channels and block nerve injury-induced hyperpathia [433]. Increased expression of the potassium channel Kv1.2 reduced nerve injury-induced hyperpathia [434]. Transfecting small interfering RNAs can be used to reduce $Na_v1.7$RNA and protein in DRG and reduce hyperpathia in diabetic rats [435]. An important issue is the ability of various viral vectors to transefect dorsal root ganglion cells vs.spinal parencyhma. After intrathecal delivery, DRG neurons are readily transfected wheras the transfection of spinal neurons appears restricted at best [436]. Issues related to the ability of viral particles to move into the parechnyma have been consideredas well as the use of direct intraparenchymal injections [437]. Here the potential role of the pia mater in establishing such a barrier to particulates and larger molecules in the CSF has been noted in humans and dogs [438-440]. Techniques to increasethat movement have been proposed,including the use of hypertonic solutions [441].

Future Issues of Intrathecal Delivery

Intrathecal Drug Distribution

The effect of intrathecal local anesthetics upon sensory input reflects their interaction with sodium channels on the sensory root (or upon superficial long tracts). Other agents exerting their action at specific receptors or certain channels, such as those for the mu opiate, GABA-B agonist, the N-type calcium channel, or agents such as substance P saporin targeting NK1 receptor-bearing cell bodies, must reach the appropriate sites to regulate the input from the root innervating a given dermatome. This required targeting poses several issues. 1) As reviewed above, these targeted sites lie largely on the terminal of the primary afferent and/or upon cells postsynaptic to the afferent input. Accordingly, at the least, we can appreciate that if these agents remain in the intrathecal sac, they, unlike local anesthetics, have an action upon coupled receptors that are present on the terminals of the axon or postsynaptic neurons. 2) Pain arising from a specific dermatome, myotome or sclerotome reflects afferent input from defined dorsal root ganglion cells. Importantly, after entry into the dorsal horn, the sensory afferent axon sends collaterals rostrally and caudally as far as 4-6 spinal segments [442, 443]. Thus, current thinking is that the input arising from any given dermatome represents an excitatory drive that is mediated in the dorsal horn not just at the spinal segment of entry, but also at levels up to several spinal segments in distance.3) Terminals of small sensory axons lie within the parenchyma (substantia gelatinosa) at depths ranging from 10-20μ (mouse and rat) to 200-300 mu in the dog and 500 mu in humans. Intrathecal (superficially applied) agents must diffuse into the parenchyma to reach these target sites. As diffusion from the surface to the presumed site of action is a time-dependent process that is proportional to distance, it is interesting to note that all things being equal for a given drug such as morphine, the time of onset is fastest in the mouse (2-3min) [444] and proportionally more delayed in larger animals (30 min in cat and dog) [445-447] to >1 hr in humans [448, 449]. Importantly, several variables define the ability of a drug to diffuse into tissue. Further, movement through lipid rich tissues is delayed for agents with low logP than for those with higher values. Thus, recording from deep dorsal horn (Lamina V) neurons in cat spinal dorsal horn, it was

demonstrated that time to onset of suppression of cell activity was greatest from morphine and least for fentanyl [450, 451], a finding that corresponds to the behavioral time course effects of these agents in preclinical models [446, 447] and clinical experience [452]. A second variable reflects on the pathways for extracellular drug movement, referred to as tortuosity and based on diffusional studies**.** Large molecules typically display a more restricted movement than small molecules [453, 454].

Accordingly, it is believed that for these membrane-targeted agents to produce a change in spinal nociceptive processing generated by a peripheral stimulus or spasticity, the intrathecally infused drug must move rostrally and caudally a distance of several segments and must then diffuse into the parenchyma to reach the membrane targets. Modeling of the CSF space has emphasized that spinal CSF does not undergo major flow [455, 456] and, thus, intrathecal agents delivered at a low rate, such as 10-20 μL/hr (as required by implanted pumps of limited reservoir volumes), undergo limited redistribution. This limited redistribution of low volume infusions has been emphasized in large animal models [38, 457-459] and to some degree inhumans [460].

Drug Dosing

A consequence of the intrinsically poor intrathecal redistribution of low volume injectates is the need to increase drug concentration to drive rostrocaudal movement. This use of high concentrations in human pumps has led to local toxicity. For opiates, the manifestation of this toxicity is the intrathecal granuloma [37, 116, 461-463]. Our group showed that the primary determinant for intrathecal opiate granulomas is the local concentration to which the tissue adjacent to the catheter tip is exposed [49, 51, 52]. Even baclofen, which has no strong evidence of granuloma formation in humans, may have limitations in that regard if concentrations higher than the currently available formulation (2mg/mL) is employed ([464] but see [267]).

Formulation

An important issue characteristic for neuraxial delivery are the formulation requirements. Typically, neuraxial formulations are water-based products with a pH between 5-7 and osmolarity in the range of 300 mOsM with minimal adjuvants (*e.g.*, surfactants, antioxidants or antimicrobial constituents) (see: [465, 466]). Few are generally regarded as safe vehicles. Surfactant/detergents (Tween, polyethylene glycol) or common solubilizing agents such as dimethylformamide or dimethylsulfoxide cannot be routinely considered as "safe" and evaluation of the safety of a spinal therapeutic employing such products must consider the potential direct tissue effects of the formulation at the specific concentrations being employed. It should also be appreciated that DMSO and other solvents can interact and solubilize various plastic-based hardware (*e.g.* catheters, syringes, tubing and plastics) used to administer the drug. The family of cyclodextrins has been widely employed preclinically for producing increased solubility and altered distribution of otherwise water insoluble agents [467-470]. Other formulation strategies, including liposome, microsphere and nanoparticle compounds, to enhance the duration of action and reduce peak concentrations of intrathecally-delivered agents have been proposed to permit single long-lasting drug delivery paradigms. Such systems have been described in *ex vivo* and *in vivo* work for baclofen [471, 472] (see [466] for review).

Infusate Delivery Profile

Enhancement of redistribution may minimize the risk of local intrathecal drug concentration-based toxicity. Strategies for enhancing redistribution and, accordingly, reducing the concentration at the catheter tip can been proposed. A solution to this issue by changing delivery of the total daily infused to multiple bolus dosing may enhance the efficacy and reduce the potential toxicity associated with intrathecal therapeutics. In previous work in the dog, continuous delivery of a given dose of morphine at a high concentration yielded granulomas. Continuous infusion of the same dose at a lower concentration did not result in a granuloma [51]. Daily bolus delivery of the same total dose of morphine had no effect [49, 447]. Other strategies that may possess at least theoretical interest is the use of repetitive microboluses instead of a truly continuous infusion and the use of multiport small orifice catheters that would evenly distribute injectate over the length of the catheter rather than from a single port at the catheter tip.

Device Compatibility

Implantable pumps require appropriate solution stability for the 4-6 weeks the pump is delivering. Stability of the solutions at body temperature and in the pump reservoir must be defined in the course of drug development. Further, formulations for continuous infusion require delivery by existing pump systems. High concentration solutions, extreme pH (*e.g.*,<3) and formulations or drug combinations may exert effects upon pumping mechanisms. Each manufacturer may have compatibility protocols that require consideration for drugs to be delivered by such devices and literature exists for some agents and agent combinations (see, for example: [473, 474]. Given the different mechanics of each pump (*e.g.*, valved vs. peristaltic), compatibility in one pumping system dose not guarantee compatibility in another.

Preclinical Assessments in Spinal Analgesic Drug Development

The rodent models have been useful in defining the pharmacology of spinal nociceptive processing. As noted above, however, the use of neuraxial drug delivery poses issues related to scaling of the volumes and kinetics of the intrathecal space. The issue of whether an intrathecal molecule can make its way to the target site in the parenchyma of humans is not readily defined in the much smaller rodent cords. This raises the need to define effects upon behavior and target engagement in a large spinal cord. Further, the FDA requirements for a drug progressing to approval mandates safety to be demonstrated in a large animal preclinical model in addition to small animal (rodent) models. Particularly with regard to pharmacokinetic profiles, large animal models such as dogs, sheep, and primates offer similar metabolic functions [458, 475]. Experimental

threshold models have been described for these species (see, for example, in dog [476-478] and in sheep [479, 480]. Such models, while useful, may not capture the complexities of the inflammatory/nerve injury pain states. Models of experimental injury such as joint injury in models of osteoarthritis are well described [481-485]. Of particular interest has been the growing implementation of clinical studies in companion animals and sheep suffering from natural pathologic processes accompanied by a pain condition. Such conditions in dogs include fore and hind limb osteosarcoma and osteoarthritis [486] and osteosarcoma [487]. Numerous scoring systems and scales are available for assessment of pain in animals, which range from simple descriptive scales, visual analogue scales, subjective verbal scales, numeric rating scale, and composite pain scales. Owner-completed questionnaires have been validated to assess the severity and impact of chronic pain syndromes in dogs with bone cancer and osteoarthritis [488]. Such inventories have been shown to be useful in defining the efficacy of a variety of spinal and [261, 384] systemic [489] therapies (see [490]. Strengths of such tools include its incorporation of animal behavior over a period of time as opposed to assessment once under the influence of an abnormal environment. Appropriate study design is essential in preclinical therapeutic assessment to ensure studies are carried out in a humane fashion with the effort to minimize the severity and duration of pain to that which is necessary for the information sought. Appropriate rescue therapeutics are incorporated into the study design as in human clinical trials to provide pre-emptive analgesia when at all possible, analgesia once the study endpoint is met or to provide rescue analgesia if pain is beyond the acceptable severity as determined by the study guidelines when pain is sustained in a study.

Intrathecal Drug Safety Evaluation

A final issue to consider in developing a spinal agent is the need for specific preclinical safety evaluations employing this route of delivery. Issues of such safety evaluations have been extensively discussed [243, 244, 462, 491]. Several specific guidelines will be outlined here.

i) At a minimum, drugs for neuraxial delivery must undergo preclinical safety assessments for the appropriate route (epidural/intrathecal) with the delivery motif proposed (*e.g.*, bolus vs. continuous infusion) in functionally relevant large animal models (dog, sheep, primate), which have been shown to display drug responses in previous work. The use of large animals vs. small animals (*e.g.*, rodent) reflects upon the issue of scaling of intrathecal volumes and kinetics across species particularly at the extremes.

ii) Study design must incorporate drug concentrations that meet or exceed those to be developed for human use and employ the respective formulation proposed for approval. Where feasible, the drug dosing should be shown to have appropriate target engagement (*e.g.*, behavioral endpoints, CSF concentrations, and knock-out of target marker (*e.g.*, receptor or enzyme protein). The development of such data is important not only because it demonstrates confirmatory effects in another species, it has the practical benefit of being able to show the safety data studies to be constructed so as to define safety as multiples of a therapeutically effective dose in a large spinal cord (see, for example [390]).

iii) In the development of chronically delivered agents, the minimum length of exposure profiles should be 1-3 months to ensure that long-term safety is demonstrated.

iv) Systemic safety must also be demonstrated for drugs or compounds administered spinally. Neuraxially delivered drugs undergo local distribution into the CSF. Flow patterns are largely influenced by respiratory and cardiovascular cycles (respirations and pulsations, respectively) with drug uptake into spinal cord tissue or into the meninges ultimately occurring. Elimination of spinally-administered drugs eventually occurs through the systemic circulation, thus systemic effects and plasma concentrations must be measured to demonstrate systemic safety.

v) The principle characteristic separating pharmacological investigation from one defining safety study is the implementation of systematic histopathology of the target tissues (see [492]). An important implication of the above commentary is that because of the peculiar nature of the intrathecal space as regards local and persistent exposure is that changes in drug delivery profile (bolus vs. infusion), volume or rate of drug delivery and most certainly formulation (pH/osmolarity/ionic or additive content) and most certainly concentration must be considered as defining variables for the characterization of potential toxicity. Our experience with alterations in these variables for intrathecally delivered drugs (*e.g.*, opiates and local anesthetics) emphasizes that such changes can have clear impacts upon the assertion of safety of the formulation.

vi) Adult versus neonate. Finally, it has long been appreciated that neuraxial anesthesia and pain management therapies are relevant to the neonatal and pediatric population. The current concerns over the effects of general anesthetics on neural development in the young population has increased the focus on the neuraxial route. Yet, there has been a paucity of data to indicate the relative safety of neuraxial anesthetics in the same population (see [196, 493-496]. Given issues of pathway development and synaptic connectivity, assessment of the effects of neuraxial agents on concurrent and future spinal function must be considered in further application of analgesics and analgesic agents to this space [491].

SUMMARY

In summary, the spinal delivery of therapeutic agents reflects upon the role played by the spinal cord in a number of sensory and motor functions and the diverse pharmacological components that are associated with these systems. The initial demonstration of spinal catheterization in the rodent provided a robust model to assess the effects of drugs with an action limited to the spinal cord on behavioral function [497, 498]. The robust and selective effects of spinal opiates on pain behavior first shown in this rodent model (and the growing understanding of the complex algorithms that encode the input-output relationships in the spinal dorsal horn has resulted in exciting advances in the management of these adverse sensations, which, upon arrival at higher centers, initiate the pain experience.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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